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Request for grant of a patent

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1. Your reference — 91208/JND

2. Patent application number *(The Patent Office will fill in this part)* — **9910158.6** — 30 APR 1999

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Cyclops Genome Sciences Limited
30 Pilgrim's Lane
London NW3 1SN

Patents ADP number *(if you know it)* 7651862001

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention ISOLATION OF NUCLEIC ACID

5. Name of your agent *(if you have one)* — Page White & Farrer

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

54 Doughty Street
London WC1N 2LS

Patents ADP number *(if you know it)* 1255003

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

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Request for preliminary examination and search (Patents Form 9/77)	No
Request for substantive examination (Patents Form 10/77)	No
Any other documents *(please specify)*	No

11. I/We request the grant of a patent on the basis of this application.

Signature PAGE WHITE & FARRER Date 30 April 1999

12. Name and daytime telephone number of person to contact in the United Kingdom: Mr J N Daniels 0171-831-7929

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ISOLATION OF NUCLEIC ACID

Field of the Invention

The present invention relates to a method for isolating RNA from a sample and to a kit for use in such a method.

Background to the Invention

The process by which RNA is separated from other molecules, in particular other cellular components such as proteins, lipids and DNA is widely known and described in the literature. It is a key process in the understanding of gene function and structure and drug development. However, due to the similar physical characteristics of RNA and DNA, RNA preparations are frequently if not always contaminated with DNA, leading to major difficulties in the analysis of results.

Currently mRNA is separated from genomic DNA by using a method based on oligo (dT) interacting with the poly A tail (Aviv and Leder., Proc. Natl. Acad. Sci. USA. 69, 1408-1412 (1972). However, A-rich DNA sequences are co-purified with this method leading to mRNA contaminated with A-rich DNA. Alternatively, nucleases that are specific for either RNA or DNA can be employed. Such highly purified enzymes are costly to use and frequently require removal before the nucleic acid can be used. For example RNase free, DNase must be removed by phenol extraction or heat inactivation otherwise it will destroy for example PCR primers or other DNA based reagents in all post-nuclease reactions. Another method is to use a mixture of phenol, chloroform, isoamyl

alcohol (50:49:1), whereby DNA preferentially partitions into the organic phase whilst RNA remains in the aqueous phase. This method at best still leaves a significant amount of DNA contaminating the RNA and hence is of limited practical use. Another method is the TRI reagent™ (Molecular Research Center, Inc) that allows the simultaneous separation of DNA, RNA and proteins. However, it requires careful separation of different phases from each other and subsequent centrifugation steps whilst not assuring complete separation of DNA from the RNA due the difficulty of pipetting small volumes of liquid without cross-contamination. Another method is anion exchange chromatography which can separate RNA from DNA but the expense and difficulty of setting up the column as well as the restriction to purifying only small nucleic acids precludes its use from the majority of laboratories.

Summary of the Invention

The present invention provides a method for isolating from a sample RNA comprising an oligo-or polynucleotide, which method comprises:

(a) treating the sample with a reactant capable of covalently modifying the 2'-OH position of the ribose rings of the RNA under conditions so that a proportion of the 2'-OH positions of the ribose rings bear a substituent; and

(b) separating material containing the substituent from the sample so as to isolate the RNA.

The RNA may be mRNA, tRNA, rRNA, viral RNA, synthetic RNA such as chemically synthesised or *in vitro* transcribed

forms, or any other form of RNA, such as hnRNA. The RNA may be a mixture of different types of RNA and may be in single- or double-stranded form and even contain internal regions of secondary structure such as is commonly found in tRNA. According to the present invention an oligonucleotide generally has a sequence of up to about 80 bases and a polynucleotide generally has a sequence length of more than about 80, preferably more than about 100 bases. A preferred length for a polynucleotide is at least 1kb.

The mRNA may or may not have a cap and/or poly A tail. The mRNA or viral RNA used in the present invention is preferably naturally-occurring. A naturally-occurring RNA according to the present invention typically comprises a nucleotide sequence which is found in nature and which generally encodes a polypeptide having biological activity, or such a nucleotide sequence which is modified, for example to alter in some way the biological activity of the polypeptide encoded thereby. Whilst the naturally-occurring RNA is preferably obtained by transcription from a suitable template, itself usually naturally-occurring, in some cases the naturally-occurring RNA can be obtained synthetically. mRNA according to the present invention does not encompass simple homopolynucleotides (poly A, poly U, poly G and poly C) which can be generated synthetically but are biologically non-functional.

An important aspect of this invention is modification of mRNA since it is of major scientific interest and serves as

a good example of the problems encountered when manipulating RNA. The invention further provides methods for obtaining intact full-length copies of mRNA and other types of RNA isolated from cellular sources that demonstrate increased stability in conditions that would otherwise destroy a major fraction of the unmodified RNA.

Modification at the 2'-OH position is preferably substantially regiospecific. Thus, there is preferably substantially no modification of the bases, phosphodiester bonds and/or any other position within the RNA chain. In this way, the polynucleotide retains important properties of the RNA. For example, advantageously, the polynucleotide is preferably modified so that a single strand of the polynucleotide is replicable by a nucleic acid polymerase to generate a second strand of polynucleotide complementary to the single strand.

The modification at the 2'-OH position may be such that the entire OH of the 2'C of the ribose ring is replaced by a reactant group R as in 2'-R or by OR having 2'-OR where the -O- group may or may not originate from the 2'-OH group. Accordingly, the substituent at the 2'-OH position in this case is R or OR respectively.

Various reactants or reactant combinations may be used, optionally in the presence of a catalyst, to provide these substituents, as described in further detail in the Examples below. Advantageously, the reactant comprises an acid anhydride, an acid chloride, a carboxylic acid or an

N-acylimidazole, each of these reactants participating in an acylation reaction with the RNA. Under these reaction conditions, the reaction medium may further comprise an acylation catalyst. For example, where the reactant comprises an acid anhyride, this may be reacted with the RNA in the presence of a catalyst such as a fluoride ion or amino pyridine. As a further example, where the reactant comprises an acid chloride or N-acylimidazole, the reactant may be reacted with the RNA in the presence of an amino pyridine. As a further example, where the reactant comprises a carboxylic acid, this may be reacted with the RNA in the presence of a dehydrating agent or a catalyst, such as an isocyanide catalyst. A preferred aminopyridine catalyst is dimethyl aminopyridine (DMAP).

The organic solvent used in the reaction medium of the present invention preferably comprises an organic base and may comprise an organic solvent in which is dissolved the organic base or, in a preferred embodiment, may be the organic base itself. It is preferred that the reactant is soluble in the organic solvent. In a preferred embodiment the reaction medium further comprises water. In this way RNA to be modified may be conveniently added to the organic solvent as an aqueous solution of RNA. Typical organic solvents include alkanes such as hexane and pentane, pyridine, acetonitrile, dimethylformamide, dichloromethane, acetone, diethyl ether, benzene, chloroform, ethyl acetate, light petroleum, tetrahydrofuran, carbon tetrachloride, dichloroethane, dioxane, carbon disulphide, nitromethane, dimethyl sulphoxide, hexamethylphosphoric triamide and

toluene. Typical organic bases include pyridine, triethylamine, trimethylamine, diisopropylethylamine, *N,N*-diethylaniline, *N,N*-dimethylaniline, 1,5-diazabicyclo (4,3,0) non-5-ene (DBN), 1,8-diazabicyclo (5,4,0) undec-7-ene (DBU) and *N*-methylmorpholine. Triethylamine $(CH_3CH_2)_3N$ is a stronger amine base than pyridine, aniline, diethylamine or trimethylamine but less so than pyrrolidone. It is one of the strongest amine bases. A preferred organic base which acts as an solvent is triethylamine (TEA). Where a catalyst is to be used, it is convenient for the catalyst to be soluble in the organic solvent as well. The water and the organic solvent may form different phases in the reaction medium. For example, the water and the organic solvent may be immiscible with one another and form phases which will separate upon standing. Where there is more than one phase, the RNA may be reacted with the reactant under conditions of phase transfer catalysis.

The amounts of water and organic solvent may be varied and will depend to some extent upon the particular organic solvent/base/catalyst system to be used. Advantageously, the reaction medium comprises at least 50% organic solvent, preferably at least 80%, more preferably at least 90% and more preferably at least 95% v/v. Typically, the ratio of water:organic solvent is in the range 1:50 to 1:10, preferably around 1:20.

In the absence of a catalyst, the reaction time is generally from 20 to 60 mins. In the presence of the

catalyst, the reaction proceeds more quickly, the reaction time generally being completed within about 20 seconds.

On a vol/vol basis it is found that the ratio of reactant to reaction medium (especially acetic anhydride triethylamine/DMAP) is preferably in the range 1:200 to 1:10, more preferably around 1:20. Too little reactant gives a partial reaction and too much makes the reaction difficult to control.

In accordance with the method of the present invention, RNA is isolated from the sample by separating material which contains the substituent which has been used to modify the 2'-OH position of the ribose rings. Accordingly, a substituent needs to be selected so as to confer upon the RNA a property which the unmodified RNA does not possess. In one aspect, the substituent comprises an affinant capable of being recognised or bound by a partner such as a partner immobilised to a solid phase, or a protein. The affinant may be a hapten such as biotin or fluorescein which could be recognised and bound by an antibody or another protein such as streptavidin. Another useful affinant comprises a primary amino group capable of chemically reacting with an immobilised partner such as succinimide.

In a preferred arrangement, the substituent comprises a hydrophobic substituent so that the RNA may be modified to render it more hydrophobic for the purpose of isolation. Both DNA and RNA are relatively hydrophilic molecules. It

is quite difficult to separate RNA from DNA because their physical characteristics are similar. By increasing the hydrophobicity of RNA relative to DNA it is possible to improve the separation of the two types of nucleic acid. This is useful for either removing contaminating RNA from a DNA sample or removing contaminating DNA from RNA. For example it is important to remove bacterial RNA from a plasmid preparation prior to restriction enzyme analysis so that small DNA fragments are not obscured by the co-migrating RNA during agarose gel electrophoresis. Alternatively it is important to remove traces of DNA such as genomic DNA or viral DNA from cellular or viral RNA. Such DNA contamination often leads to false positives following RT-PCR amplification.

The hydrophobic substituent typically comprises a substituent, OR, wherein R comprises C_1-C_{36} alkyl; C_1-C_{36} alkenyl; C_1-C_{36} alkynyl; C_1-C_{36} haloalkyl; C_1-C_{36} aminoalkyl; C_1-C_{36} alkoxyalkyl; C_1-C_{36} haloalkoxyalkyl; C_1-C_{36} aminoalkoxyalkyl; C_6-C_{36} aryl; C_6-C_{36} alkylaryl; C_6-C_{36} arylalkyl; C_6-C_{36} arylalkenyl; C_1-C_{36} alkanoyl; C_1-C_{36} alkenoyl; C_1-C_{36} haloalkenoyl; C_1-C_{36} aminoalkanoyl; C_6-C_{36} arylalkanoyl; C_6-C_{36} arylalkenoyl; C_6-C_{36} aryloxyalkanoyl; C_6-C_{36} alkylarylalkanoyl; C_6-C_{36} haloarylalkanoyl; C_6-C_{36} aminoarylalkanoyl; C_1-C_{36} alkylsilanyl or C_{12}-C_{28} diarylphosphano; or a substituent R', wherein R' comprises C_1-C_{36} alkyl; C_1-C_{36} alkenyl; C_1-C_{36} alkynyl; C_1-C_{36} haloalkyl; C_1-C_{36} aminoalkyl; halo; amino; C_1-C_{36} alkylamino; C_6-C_{36} aryl; C_1-C_{36} alkylaryl or C_1-C_{36} arylalkyl.

In one embodiment, the hydrophobic substituent comprises a C_4-C_7 carbon chain or ring, preferably one which arises where the reactant comprises butyric anhydride, pentanoic anhydride, hexanoic anhydride or benzoic anhydride. In this case, it is preferred that the proportion of 2'-OH groups bearing the substituent is at least 10%.

Alternatively, the hydrophobic substituent may comprise a C_8-C_{12} carbon chain or ring in which case it is preferred that the proportion of 2'OH positions bearing the substituent is in the range 1 to 10%. As a further alternative, the hydrophobic substituent may comprise a C_{12}-C_{36} carbon chain or ring more preferably a C_{12} to C_{24} carbon chain or ring. In this case, it is preferred that the proportion of 2'-OH positions bearing the substituent is up to 1%. Thus, whilst modification of up to 1% may provide a sufficiently hydrophobic modified RNA where a long chain length of substituent is used, substituents of lower carbon chain length require a higher percentage, perhaps in the range up to 95%, to be effective.

A number of methods for isolating RNA from samples treated in accordance with the present invention exist. For example, the step (b) of separating material containing the substituent from the sample may comprise contacting the treated sample from step (a) with a hydrophobic solid phase so as to bind the material containing the hydrophobic substituent and optionally washing the material bound to the solid phase. The solid phase preferably comprises hydrophobic particles. This method may further comprise a

step of eluting the material bound to the solid phase by treating with a detergent, a chaotrope or a solvent, by lowering the salt concentration or by cleaving the substituent from the 2'-OH position of the ribose rings.

In a further embodiment, step (b) comprises treating the treated sample from step (a) with a lyotrophic salt to aggregate the material containing the hydrophobic substituent as an RNA precipitate, and isolating the precipitate. The lyotrophic salt preferably comprises ammonium sulphate, an alkali metal chloride, magnesium chloride or calcium chloride.

In a further embodiment, step (b) may comprise treating the treated sample with a non-polar solvent to form the hydrophobic liquid phase which contains the material containing the hydrophobic substituent, and isolating the hydrophobic liquid phase. The non-polar solvent typically comprises pentane, cyclohexane, toluene, benzene, light petroleum, xylene or hexane.

In a further aspect, the present invention provides a kit for isolating from a sample RNA comprising an oligo- or polynucleotide, which kit comprises:

(i) a reaction system for modifying the RNA to form a modified oligo- or poly-nucleotide in which a proportion of the 2'-OH positions of the ribose rings bear a substituent; and

(ii) a separation system for separating material containing the substituent from the sample, so as to isolate the RNA.

Preferably, the reaction system comprises:

(a) an organic solvent; and

(b) a reactant capable of covalently modifying the 2'-OH position of the ribose rings of the RNA in the presence of the organic solvent. The organic solvent and reactant are discussed in further detail above.

The present invention will now be described in further detail, by way of example only, with reference to the following examples.

General Procedure

A complex mixture of DNA and RNA polynucleotides from a cellular source is treated with a lipophillic acid anhydride such as butyric anhydride ($C_8H_{14}O_3$), pentanoic anhydride ($C_{10}H_{18}O_3$), hexanoic anhydride ($C_{12}H_{22}O_3$), heptanoic anhydride ($C_{14}H_{26}O_3$) or benzoic anhydride ($C_{14}H_{10}O_3$) as described. Longer chain lengths than heptanoic anhydride are not preferred because incomplete modification of the RNA chain has been observed, possibly due to steric hindrance between the RNA chain and the long chain anhydrides. Such reagents will modify the single terminal 3'-OH group and possibly the single 5'-OH or phosphate group of the DNA or RNA chain but all or substantially all the 2'-OH groups of the RNA, thereby strongly altering the physical characteristics of the RNA towards lipophilicity. However the DNA is modified at only one (3'-OH) or a maximum of two (one 5'-OH/phosphate and one 3'-OH) position(s) and therefore remains overall hydrophilic.

This difference in lipophilicity then serves as the basis for separation of the DNA from RNA.

The hydrophobic RNA is preferably manipulated with silanised plasticware and/or with detergents such as 0.01% Tween-20, 0.01% NP40 or 0.1 Triton X-100 so that sample loss is reduced.

The capture surface or material for the modified RNA could be encapsulated within a centrifuge device such as a microcentrifuge spin-column or tube insert. Alternatively, it could be encapsulated in a pipette tip such as those commonly used to measure 200µl or 1 ml volumes. In either case, capture, washing and elution of the modified RNA is improved because the time required to separate the modified RNA from the contaminants is reduced.

Multiple RNA samples could be purified in parallel by the use of devices bearing multiple capture surfaces. An example would be a 96-well plate whereby each well is hydrophobic and suitable for capture, washing and elution of one modified RNA sample. A further example would be a vessel or chamber suitable for the modification reaction and in addition the capture, washing and elution of the RNA sample. In this way, both the modification and purification is carried out in the same vessel or chamber improving sample throughput and productivity. The modification, capture, washing and elution of the RNA sample could be automated and involve robots.

Methods of treatment of the RNA are described in further detail in copending UK patent applications, nos. (PWF 91205 entitled POLYNUCLEOTIDES, 91206 entitled POLYNUCLEOTIDES and 91207 also entitled POLYNUCLEOTIDES). Each of these copending patent applications was filed on the same day as the present application in the name of the same applicant. For example, Example 6 of each of these copending patent applications sets out one preferred method of modifying RNA involving a DMAP-catalysed acetylation reaction. The methodology in that Example may be modified using instead of acetic anhydride, anhydrides of longer chain length including butyric or pentanoic anhydrides as set out in Example 54 of each of the copending applications. In addition, Example 1 of each of these copending patent applications sets out a method of modifying a total cellular RNA population and selection of the mRNA fraction.

It will also be apparent to those skilled in the art that a crude cellular or tissue lysate consisting of RNA, DNA, protein and lipids etc. may serve as the sample for the modification reaction. In this case, the reactant may modify not only the 2'-OH group of the RNA chain but also the hydroxyl bearing side chains of the amino acids tyrosine, serine or threonine of proteins. It will be apparent that such a reaction is beneficial because it will lead to the deactivation of cellular nucleases and therefore allow the selection of RNA in a more intact form. Furthermore, cells could be disrupted in the presence of the reactant so that nucleases are immediately inactivated on release from the cell and the RNA is immediately

modified and therefore protected from any remaining nucleases. In this latter case, increased concentrations of the reactant in the reaction may be required in order to inactivate the nucleases fully as well as to modify fully the 2'-OH groups of the RNA sample.

The treated DNA and RNA sample in a high salt buffer (e.g. 1 - 5 M ammonium sulphate, preferably 10 mM phosphate, pH 7.0, 1.5 M ammonium sulphate) is passed over a hydrophobic column as is commonly used for reverse phased liquid chromatography. For example reversed phase packings based on silica may have bonded hydrocarbon chains of C4, C8 or C18 or others may be based on polystyrene (e.g. POROS® and Oligo R3, PerSeptive Biosytems, USA) may be used under appropriate conditions. Further alternatives could include plastics such as polypropylene, polycarbonate, polyvinylidene fluoride (manufactured under the tradename Hydrophobic Durapore, Millipore, USA) an PTFE. With all plastics those with a large surface area to weight are preferred in order to increase the effective hydrophobic binding surface. Such surfaces exist as beads of cellulose covered with butyl, octyl or phenyl groups (Cellufine Butyl, Cellufine Phenyl and Cellufine Octyl, Amicon, Mass, USA) and are well known in the art. Other bead compositions could include silica matrix modified with polyethylene-imine (Si PAE, Amicon, Mass, USA). After washing extensively with water to remove all traces of DNA, the RNA can be released by several methods. Firstly and preferably, the RNA can be deprotected by cleavage of the modifying group with 50% ammonia treatment, KCN in 95%

EtOH, K_2CO_3 in aqueous methanol or other conditions which are known to lead to the cleavage of the ester linkage (see Protective Groups In Organic Chemistry, 2nd edition, Ed. T.W. Greene, Wiley-Interscience). The RNA can then be collected and purified further if required by, for example oligo (dT) selection of the mRNA.

Alternatively the RNA can be removed from the solid support in its modified form using detergents such as 0.1% SDS, 0.002M Triton X-100™ (Union Carbide Chemical and Plastics, Inc.), Nonidet P-40™ (Shell Oil Co.) or 1% TWEEN™ (ICI Americas, Inc.) in aqueous solution. Or alternatively by lowering the salt concentration (preferably 10 mM phosphate, pH 7.0) in the binding buffer to a point where elution is brought about. Another approach would include the addition of a chaotropic agent such as ethylene glycol, urea, guanindine-HCl or thiocyanate salts. Solvents such as isopropanol, methanol, ethanol, DMSO or acetronitrile may also be used to elute the RNA up to a final concentration of 40% (v/v) in water.

Example 1

Use of lyotrophic salts

Alternatively, separation can be brought about using such commonly used salts as ammonium sulphate or sodium chloride (known as « lyotrophic salts ») which are routinely used for separating proteins with varying hydrophobic properties from solution by a process known as 'salting out'. Proteins with the greatest hydrophobic tendencies aggregate and precipitate out of solution at the lowest lyotrophic

salt concentration. By comparison with this system it will be apparent that a specific salt concentration can be selected that will cause modified RNA to aggregate whilst the hydrophilic DNA will remain in solution thereby providing the basis for separation. The specific salt concentration will depend on the particular modifying reagent used. For example, RNA modified with longer carbon chains which are therefore more hydrophobic such as heptanoic anhydride will aggregate at a lower salt concentration than those modified by shorter chain lengths such as acetic or propionic anhydride. Other salts that may be used (in order of decreasing ability to cause RNA to aggregate) include RbCl, KCl, NaCl, CsCl, LiCl, $MgCl_2$ and $CaCl_2$.

Aggregates can be collected either by allowing precipitation to occur at unit gravity or by centrifugation at 1-5000 g in a bench top centrifuge. The modified RNA can then be collected and salts removed by washing with several washes of 70% ethanol. Alternatively, salts can be removed by using a Centricon-50 column (Amicon, MA, USA) and washing with a either water or 0.1% SDS. Following this purification the RNA may be used as a template for RT-PCR or in Northern blotting.

Example 2

Partition Between Two Solvents

As another alternative, treated RNA with increased hydrophobic properties could be simply purified from a complex mixture of proteins, DNA and other cellular

components by mixing and agitating by use of a vortex etc. the mixture with appropriate solvents such as (in order of decreasing polar property) pentane, toluene, chloroform, THF, DMSO or methanol. Under ideal conditions it would be expected that all the modified RNA would partition into the hydrophobic phase whilst non-RNA contaminants would remain in the aqueous phase. Simple separation of the hydrophobic phase by pipetting followed by ethanol precipitation or evaporation of the solvent would provide a highly purified source of modified RNA.

Example 3

Differential Interaction with Immobilised hydrocarbon chains

Direct interaction between RNA molecules bearing hydrophobic groups and hydrophobic groups attached to a solid support would provide an efficient means to separate RNA from contaminants. Hydrophobic solid supports include ethyl-, propyl-, butyl-, pentyl-, hexyl-, octyl-, decyl and dodecyl-agarose affinity chromatography media (Catalogue ref. AAF-8, Sigma-Aldrich Chemicals). Interaction between the modified RNA and the media should be stronger with longer chain lengths attached to either the RNA or immobilised support. By careful choice of the binding solution it is possible to selectively bind RNA to the beads whilst the contaminants such as DNA are retained in the binding solution. The polarity of the solvent used for binding and washing, the type and concentration of detergent, temperature of interaction and carbon chain

length used will all influence the effectiveness of the purification.

Release of the modified RNA from the solid support is effectuated by the addition of;

1) a deprotecting agent such as ammonia that leads to carbon chain cleavage and therefore separation of the RNA from the hydrophobic groups,

2) a solvent with a low polarity such as pentane or toluene that will preferentially bind to the hydrocarbon side chains of the RNA and thereby disrupt the hydrophobic interaction with the solid support, or

3) a detergent such as SDS or TWEEN that is capable of disrupting the hydrophobic interaction with the solid support.

CLAIMS:

1. A method for isolating from a sample RNA comprising an oligo-or polynucleotide, which method comprises:
(a) treating the sample with a reactant capable of covalently modifying the 2'-OH position of the ribose rings of the RNA under conditions so that a proportion of the 2'-OH positions of the ribose rings bear a substituent; and
(b) separating material containing the substituent from the sample so as to isolate the RNA.

2. A method according to claim 1, wherein step (a) is carried out in a reaction medium which comprises an organic solvent.

3. A method according to claim 2, wherein the organic solvent comprises an organic base.

4. A method according to claim 2 or claim 3, wherein the reactant comprises an acid anhydride, an acid chloride, a carboxylic acid or an N-acylimidazole.

5. A method according to claim 4, wherein the reaction medium further comprises an acylation catalyst.

6. A method according to any one of claims 2 to 5, wherein the reaction medium further comprises water.

7. A method according to any one of the preceding claims, wherein the RNA comprises mRNA or viral RNA.

8. A method according to any one of the preceding claims, wherein the sample comprises a sample from a cell or blood.

9. A method according to any one of the preceding claims, wherein the sample includes DNA.

10. A method according to any one of the preceding claims, wherein the substituent comprises a hydrophobic substituent.

11. A method according to claim 10, wherein the hydrophobic substituent comprises a substituent, OR, wherein R comprises C_1-C_{36} alkyl; C_1-C_{36} alkenyl; C_1-C_{36} alkynyl; C_1-C_{36} haloalkyl; C_1-C_{36} aminoalkyl; C_1-C_{36} alkoxyalkyl; C_1-C_{36} haloalkoxyalkyl; C_1-C_{36} aminoalkoxyalkyl; C_6-C_{36} aryl; C_6-C_{36} alkylaryl; C_6-C_{36} arylalkyl; C_6-C_{36} arylalkenyl; C_1-C_{36} alkanoyl; C_1-C_{36} alkenoyl; C_1-C_{36} haloalkenoyl; C_1-C_{36} aminoalkanoyl; C_6-C_{36} arylalkanoyl; C_6-C_{36} arylalkenoyl; C_6-C_{36} aryloxyalkanoyl; C_6-C_{36} alkylarylalkanoyl; C_6-C_{36} haloarylalkanoyl; C_6-C_{36} aminoarylalkanoyl; C_1-C_{36} alkylsilanyl or C_{12}-C_{28} diarylphosphano; or a substituent R', wherein R' comprises C_1-C_{36} alkyl; C_1-C_{36} alkenyl; C_1-C_{36} alkynyl; C_1-C_{36} haloalkyl; C_1-C_{36} aminoalkyl; halo; amino; C_1-C_{36} alkylamino; C_6-C_{36} aryl; C_1-C_{36} alkylaryl or C_1-C_{36} arylalkyl.

12. A method according to claim 11, wherein the hydrophobic substituent comprises a C_4 to C_7 carbon chain or ring.

13. A method according to claim 12, wherein the reactant comprises butyric anhydride, pentanoic anhydride, hexanoic anhydride or benzoic anhydride.

14. A method according to claim 12 or claim 13, wherein the proportion of 2'-OH positions bearing the substituent is at least 10%.

15. A method according to claim 11, wherein the hydrophobic substituent comprises a C_8-C_{12} carbon chain or ring.

16. A method according to claim 15, wherein the proportion of 2'-OH positions bearing the substituent is in the range 1 to 10%.

17. A method according to claim 11, wherein the hydrophobic substituent comprises a C_{12}-C_{36} carbon chain or ring.

18. A method according to claim 17, wherein the proportion of 2'-OH positions bearing the substituent is up to 1%

19. A method according to any one of claims 10 to 18, wherein step (b) comprises contacting the treated sample from step (a) with a hydrophobic solid phase so as to bind

the material containing the hydrophobic substituent and optionally washing the material bound to the solid phase.

20. A method according to claim 19, wherein the solid phase comprises hydrophobic particles.

21. A method according to claim 19 or claim 20, which further comprises a step of eluting the material bound to the solid phase by treating with a detergent, a chaotrope or a solvent, by lowering the salt concentration or by cleaving the substituent from the 2'-OH position of the ribose rings.

22. A method according to any one of claims 10 to 21, wherein step (b) comprises treating the treated sample from step (a) with a lyotrophic salt to aggregate the material containing the hydrophobic substituent as an RNA precipitate, and isolating the precipitate.

23. A method according to claim 22, wherein the lyotrophic salt comprises ammonium sulphate, an alkali metal chloride, magnesium chloride or calcium chloride.

24. A method according to any one of claims 10 to 18, wherein step (b) comprises treating the treated sample with a non-polar solvent to form a hydrophobic liquid phase which contains the material containing the hydrophobic substituent, and isolating the hydrophobic liquid phase.

25. A method according to claim 24, wherein the non-polar solvent comprises pentane, cyclohexane, toluene, benzene, light petroleum, xylene or hexane.

26. A kit for isolating from a sample RNA comprising an oligo- or polynucleotide, which kit comprises:
(i) a reaction system for modifying the RNA to form a modified oligo- or poly-nucleotide in which a proportion of the 2'-OH positions of the ribose rings bear a substituent; and
(ii) a separation system for separating material containing the substituent from the sample, so as to isolate the RNA.

27. A kit according to claim 26, wherein the reaction system comprises:
(a) an organic solvent; and
(b) a reactant capable of covalently modifying the 2'-OH position of the ribose rings of the RNA in the presence of the organic solvent.

28. A kit according to claim 27, wherein the organic solvent comprises an organic base.

29. A kit according to claim 27 or claim 28, wherein reactant comprises an acid anhydride, an acid chloride, a carboxylic acid or an N-acylimidazole.

30. A kit according to claim 29, which further comprises an acylation catalyst.

31. A kit according to any one of claims 27 to 30, wherein the substituent comprises a hydrophobic substituent.

32. A kit according to claim 31, wherein the hydrophobic substituent comprises a substituent, OR, wherein R comprises C_1-C_{36} alkyl; C_1-C_{36} alkenyl; C_1-C_{36} alkynyl; C_1-C_{36} haloalkyl; C_1-C_{36} aminoalkyl; C_1-C_{36} alkoxyalkyl; C_1-C_{36} haloalkoxyalkyl; C_1-C_{36} aminoalkoxyalkyl; C_6-C_{36} aryl; C_6-C_{36} alkylaryl; C_6-C_{36} arylalkyl; C_6-C_{36} arylalkenyl; C_1-C_{36} alkanoyl; C_1-C_{36} alkenoyl; C_1-C_{36} haloalkenoyl; C_1-C_{36} aminoalkanoyl; C_6-C_{36} arylalkanoyl; C_6-C_{36} arylalkenoyl; C_6-C_{36} aryloxyalkanoyl; C_6-C_{36} alkylarylalkanoyl; C_6-C_{36} haloarylalkanoyl; C_6-C_{36} aminoarylalkanoyl; C_1-C_{36} alkylsilanyl or C_{12}-C_{28} diarylphosphano; or a substituent R', wherein R' comprises C_1-C_{36} alkyl; C_1-C_{36} alkenyl; C_1-C_{36} alkynyl; C_1-C_{36} haloalkyl; C_1-C_{36} aminoalkyl; halo; amino; C_1-C_{36} alkylamino; C_6-C_{36} aryl; C_1-C_{36} alkylaryl or C_1-C_{36} arylalkyl.

33. A kit according to claim 32, wherein the hydrophobic substituent comprises a C_4 to C_7 carbon chain or ring.

34. A kit according to claim 33, wherein the reactant comprises butyric anhydride, pentanoic anhydride, hexanoic anhydride or benzoic anhydride.

35. A kit according to claim 33 or claim 34, wherein the proportion of 2'-OH positions bearing the substituent is at least 10%.

36. A kit according to claim 31, wherein the hydrophobic substituent comprises a C_8-C_{12} carbon chain or ring.

37. A kit according to claim 36, wherein the proportion of 2'-OH positions bearing the substituent is in the range 1 to 10%.

38. A kit according to claim 31, wherein the hydrophobic substituent comprises a C_{12}-C_{36} carbon chain or ring.

39. A kit according to claim 38, wherein the proportion of 2'-OH positions bearing the substituent is up to 1%

40. A kit according to any one of claims 31 to 39, wherein the separation system comprises a hydrophobic solid phase for binding the material containing the substituent.

41. A kit according to claim 40, wherein the solid phase comprises hydrophobic particles.

42. A kit according to claim 40 or claim 41, wherein the separation system further comprises an elution medium for eluting RNA bound to the solid phase.

43. A kit according to any one of claims 31 to 39, wherein the separation system comprises a lyotrophic salt for aggregating the material containing the hydrophobic substituent.

44. A kit according to any one of claims 31 to 39, wherein the separation system comprises a non-polar solvent for forming a hydrophobic liquid phase which contains the material containing the hydrophobic substituent.